Chromosomal Rearrangements and Evolution of Recombination: Comparison of Chiasma Distribution Patterns in Standard and Robertsonian Populations of the House Mouse

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ABSTRACT

The effects of chromosomal rearrangements on recombination rates were tested by the analysis of chiasma distribution patterns in wild house mice. Males and females of two chromosomal races from Tunisia differing by nine pairs of Robertsonian (Rb) fusions (standard all-acrocentric, $2N = 40$ and $2N = 22$) were studied. A significant decrease in chiasma number (CN) was observed in Rb mice compared to standard ones for both sexes. The difference in CN was due to a reduction in the number of proximal chiasmata and was associated with an overall more distal redistribution. These features were related to distance of chiasmata to the centromere, suggesting that the centromere effect was more pronounced in Rb fusions than in acrocentric chromosomes. These modifications were interpreted in terms of structural meiotic constraints, although genic factors were likely involved in patterning the observed differences between sexes within races. Thus, the change in chromosomal structure in Rb mice was associated with a generalized decrease in recombination due to a reduction in diploid number, a lower CN, and a decrease in the efficiency of recombination. The effects of such modifications on patterns of genic diversity are discussed in the light of models of evolution of recombination.

MEIOTIC recombination regulates the transmission of genetic information through the segregation of chromosomes and the exchange of genic material (Korol *et al.* 1994). The latter is physically mediated by chromosomes, the number, size, morphology, and composition of which may thus contribute to determine levels and patterns of genic exchange and diversity. This occurs through two processes: inter- and intrachromosomal recombination. Interchromosomal recombination consists of the independent assortment of homologously paired chromosomes leading to the production of haploid gametes, the diversity of which is proportional to the number of chromosomes (Dutrillaux 1986). Intrachromosomal recombination involves exchange events between homologous pairs of chromosomes occurring through the formation of chiasmata, which have long been recognized cytogenetically (Mather 1938), but only recently molecularly (Anderson *et al.* 1999). Both of these components of recombination affect the rate of accumulation of deleterious mutations and the level of diversity (Nordborg *et al.* 1996; Antezana and Hudson 1997). However, according to Burt (2000), changes in the number of crossover events have a larger effect on genetic variability than those modifying diploid number.

Intrachromosomal recombination patterns can be modified by a change in chiasma rates and/or a change in the location of crossover events along the chromosome (Korol *et al.* 1994). Chiasma number depends on chromosome size (Kaback *et al.* 1992; Kaback 1996) and form, *i.e.*, one-armed or biarmed chromosomes. Chromosome morphology, in particular, determines the minimum number of chiasmata per chromosome, since proper disjunction of chromosomes requires the presence of at least one chiasma per chromosomal arm (John 1990; Paliulis and Nicklas 2000). The importance of this requirement for correct segregation has received experimental support (Koehler *et al.* 1996; Hassold *et al.* 2000) and its relation to chromosomal arms rather than to whole chromosomes has been emphasized in mammals (Dutrillaux 1986; Qumsiyeh 1994; Pardo-Manuel de Villena and Sapienza 2001). Regarding changes in location, the distribution of crossover events along chromosomes is known to be nonrandom (John 1990; Kaback *et al.* 1992; Nachman and Churchill 1996; True *et al.* 1996) and controlled by factors such as the centromere-telomere polarity of chromosomes (Ashley *et al.* 1993; Choo 1998), DNA sequence content (John and King 1985; Eyre-Walker 1993), conformation and homology (Zickler and Kleckner 1999), chiasma interference (Lawrie *et al.* 1995;

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Gorlov and Gorlova 2001), and sex (Hawley *et al.* 1993; Hassold *et al.* 2000).

As karyotypic evolution proceeds by modification of the number, structure, and composition of chromosomes, chromosomal change may immediately affect rates and patterns of recombination and, thus, the amount and distribution of genic exchanges (Trickett and Butlin 1994; Burt 2000; Rieseberg 2001). Qumsiyeh (1994) in particular has argued that chromosome rearrangements may have been selected to modulate levels of recombination. Different types of chromosomal rearrangements exist: fusions, fissions, reciprocal translocations, inversions, and heterochromatin additions and deletions (King 1993). The influence of several of these modifications in genome structure on recombination patterns has been studied in varied organisms (Coates and Shaw 1982; John and King 1985; Hale 1986; Parker 1987; Rowell 1991; Reed *et al.* 1992; Colombo 1993), but studies have focused for a major part on the effects of chromosomal heterozygosity, very few attempting to correlate chromosomal morphology with recombination rates. Results showed that changes in recombination rates varied according to the type and age of the rearrangements and extended in some cases to the structurally unchanged complement of the karyotype (Hewitt 1967; Arana *et al.* 1990). These studies have relied on the analysis of the segregation of genetic variants and/or the cytogenetic observation of chiasmata in metaphase preparations of meiotic chromosomes. Although discrepancies between estimates of recombination may be present between the two approaches and related to biases inherent to each method (Nilsson *et al.* 1993; Gill *et al.* 1997; Hassold *et al.* 2000; King *et al.* 2002), the number and localization of chiasmata are considered as accurate indicators of the rates and patterns of recombination events in mammals (Kanda and Kato 1980; Tease and Jones 1995; Nachman and Churchill 1996; Anderson *et al.* 1999).

The aim of this article is to estimate changes in the rate and distribution of recombination due to the occurrence of Robertsonian (Rb) fusions in wild populations of the house mouse, *Mus musculus domesticus*. Males and females from two chromosomal races differing by nine pairs of Rb fusions ($2N = 40$ and $2N = 22$; Nachman and Searle 1995) are studied by cytogenetical observation of chiasmata. The advantage of this experimental model is threefold. First, Rb fusions are the most widespread chromosomal rearrangement in mammals (Qumsiyeh 1994) and consist of the fusion by the centromere of two acrocentric chromosomes. These rearrangements reduce the diploid number while leaving the arm number and genic organization unchanged (King 1993). Second, chromosomal differentiation in this taxon occurred very recently (<5000 years; Auffray 1993) with little genetic divergence (Britton-Davidian *et al.* 1989; Saïd and Britton-Davidian 1991) allowing us to assess changes more related to chromosome than to genic structure. Third, the large difference in diploid number between the two chromosomal races provides the opportunity to analyze global recombination patterns in relation to extensive structural changes. The comparison of chiasma rates between these chromosomal races will allow us to test the effects of homozygous Rb fusions on recombination. In particular, as the number of chromosomal arms is not modified, intrachromosomal recombination should not be affected, although several studies reported a decrease in chiasma rates related to Rb fusions (Cattanach 1978; Davisson and Akeson 1993; Bidau *et al.* 2001; Castiglia and Capanna 2002). Finally, evolutionary interpretations of our results are discussed in the light of theoretical models of the evolution of recombination.

TABLE 1

Number of individuals (cells) analyzed by sex, race, and locality

	Females		Males	
Locality	$2N = 22$	$2N = 40$	$2N = 22$	$2N = 40$
Monastir	4 (6)		6 (84)	2 (48)
Kairouan		21 (51)		8 (147)
Djemmal	22 (49)		4 (145)	

MATERIALS AND METHODS

Samples: Twenty males and 47 females belonging to three laboratory-bred strains of wild mice from Tunisia were analyzed (Table 1). The founder mice were trapped in 1995 and 1996 in Djemmal, Monastir, and Kairouan (Chatti *et al.* 1999). The mice from these localities belong to two chromosomal races: standard mice with $2N = 40$ (Kairouan and Monastir) and Robertsonian (Rb) ones with $2N = 22$ (Djemmal and Monastir) due to the fixation of nine pairs of Rb fusions (Saïd and Britton-Davidian 1991): Rb(1.11), Rb(2.16), Rb(3.12), Rb(4.6), Rb(5.14), Rb(7.18), Rb(8.9), Rb(10.17), and Rb(13.15).

Chromosomal preparations: Mice between 41 and 698 days old were killed by cervical dislocation. Ovaries were extracted from females and cultured for 4 hr in an incubator at 37°, following the methods of Henderson and Edwards (1968) and Quinn *et al.* (1982) for the M2 culture medium. Metaphase I chromosomes were prepared following the method of Tarkowski (1966). Fifty-five cells from 26 Rb females and 51 cells from 21 standard mice were analyzed (Table 1). Testes were removed from males and meiotic chromosome preparations were obtained using the air-drying method (Evans *et al.* 1964). At least 10 spermatocytes at the metaphase I stage were recorded per male. A total of 195 cells from 10 mice with $2N = 40$ and 229 cells from 10 mice with $2N = 22$ were analyzed (Table 1). All chromosomal preparations were stained using a slightly modified C-banding protocol (Sumner 1972) to locate centromeres (Figure 1). Observations were performed using a Zeiss Axiophot microscope at ×1250 magnification and analyzed and archived with the Genevision system (Applied Imaging, Santa Clara, CA).

Chiasma analysis: In standard mice, meiotic preparations typically showed 20 acrocentric bivalents, whereas only 11 were

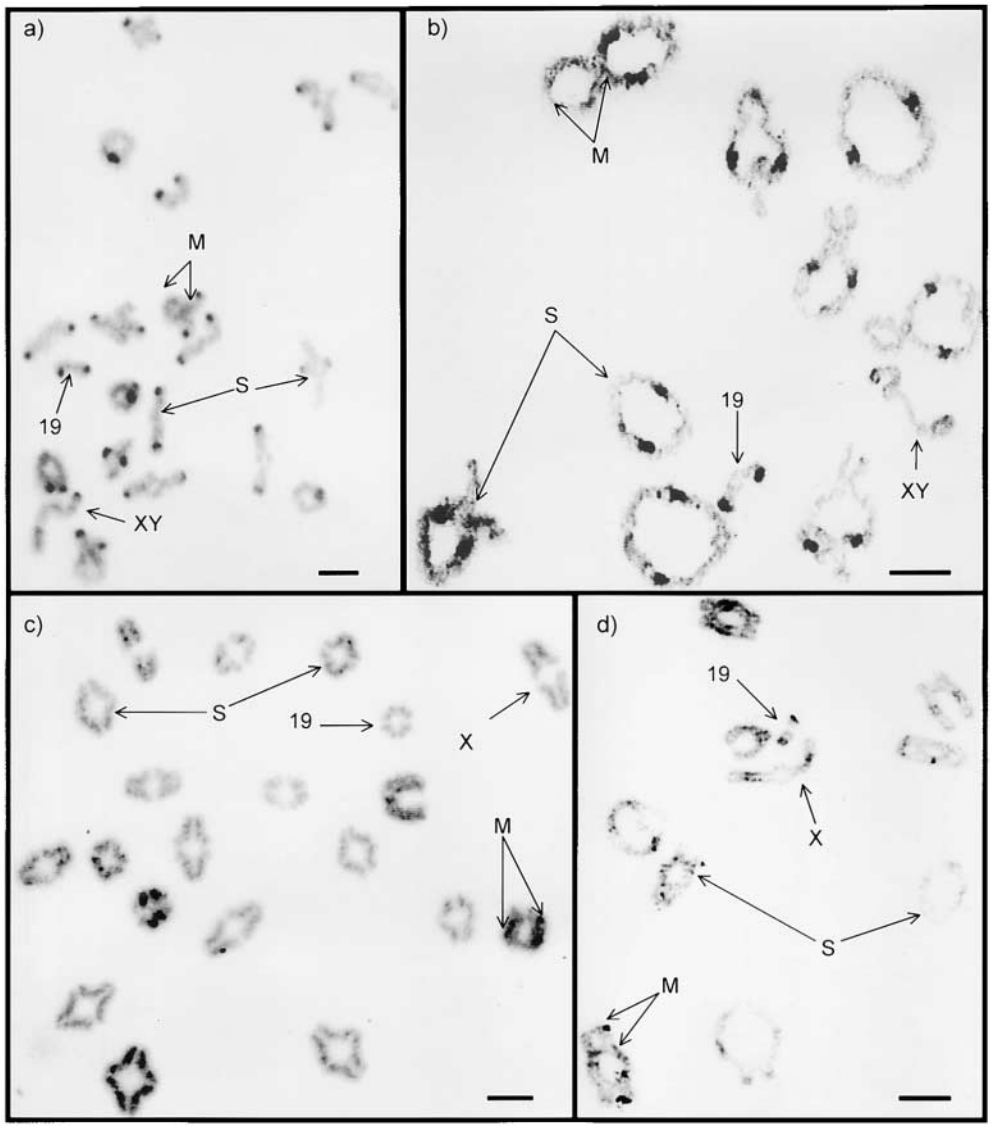

FIGURE 1.—C-banded diakinesis/metaphase-I gametocytes of standard (a) and Rb males (b) and of standard (c) and Rb females (d). S, single chiasmata; M, multiple chiasmata. Centromeres appear darkly stained. Chromosome pair 19 is indicated in standard mice (a and c) and the X bivalent in the standard female (c). Bars, 5 μm.

present in Rb individuals, consisting of 9 biarmed Rb bivalents, 1 autosomal acrocentric bivalent (chromosome 19), and the sex bivalent (Figure 1). Since centric fusions do not alter the number of chromosomal arms, both races have the same fundamental number (NF = 40). To homogenize the data and allow for comparisons between the two races, chiasmata were scored per chromosomal arm and not per chromosome in each bivalent. As individual chromosomes were not identified in the meiotic metaphases, the total number of chiasmata was counted per cell and the mean number per individual was calculated. The data were thus standardized and did not take into account the size differences between chromosomal arms. Chiasmata were separated into two types: single when only one chiasma occurred on the bivalent arm and multiple when two or more chiasmata per bivalent arm were present (Figure 1). The position of chiasmata was measured relative to the length of each bivalent arm, starting from the centromere, and was thus scored as a percentage. Measurements were made independently along the two chromatids per chromosomal arm and averaged. To evaluate measurement error, an ANOVA was calculated on a subsample of 120 bivalent arms from each chromosomal race (60 per sex). Among these, the 30 shortest and the 30 longest bivalents were discriminated. Measurement error accounted for 0.26–1.23% of the total variance in location of chiasmata, short bivalents having the highest error fractions (range 0.61–1.23%). The overall low values of measurement error justified dividing chromosomal arms into 10 segments of equal size to estimate the mean distribution of chiasmata along chromosomal arms for each race and sex. On the basis of these values, two classes were defined: nonterminal (0–90%) and terminal (>90%) chiasmata. Chiasma interference (MATHER 1938) was determined by measuring the mean distance between two chiasmata occurring on the same chromosomal arm. In addition, interference across the centromere was examined in Rb mice by comparing the distance to the centromere of the most proximal chiasmata on the two chromosomal arms of each fusion (COLOMBO and JONES 1997; BROMAN and WEBER 2000). Correlations were calculated and tested for five between-chiasma distance classes ranging from 40 to 80%.

Two chromosome pairs were singled out for a comparative analysis. The first one, chromosome 19, is the smallest in the mouse genome and the only one in an acrocentric form in the Rb sample. Its identification in standard karyotypes was performed by measuring the four smallest bivalents and assigning chromosome 19 to the shortest one. The second pair consisted of the sex chromosome bivalent, which can be unambiguously recognized in all males because of its asymmetric

TABLE 2

Mean number of chiasmata per cell per individual (± standard error)

	Females	Males	F *vs.* M
	With sex chromosomes		
Standard mice ($2N = 40$)	25.95 (±1.96)	23.96 (±1.62)	−2.52*
Rb mice ($2N = 22$)	23.56 (±1.47)	21.25 (±0.22)	−3.73***
40 *vs.* 22	−3.98***	−3.78***	
	Without sex chromosomes		
Standard mice ($2N = 40$)	24.39 (±1.79)	22.96 (±1.62)	−1.86 ns
Rb mice ($2N = 22$)	22.31 (±1.52)	20.25 (±0.22)	−3.66***
40 *vs.* 22	−3.59***	−3.78***	

Comparisons between sexes and races using Mann-Whitney tests are indicated. *Z*-values with corrected levels of significance are provided as follows: *$P < 0.05$; ***$P < 0.001$; ns, $P > 0.05$. F, females; M, males. Values including and excluding sex chromosomes are provided (see text for explanation).

form, as well as in Rb females since it is the largest acrocentric bivalent present (Figure 1). However, as no specific features discriminated the X bivalent from other similarly sized chromosomes in standard female mice, the procedure used by Lawrie *et al.* (1995) was followed to presumptively assign a bivalent to the X chromosome. These authors determined that the X bivalent identified with a specific probe corresponded to the fourth largest in size in the female meiotic karyotype. By measuring the four largest bivalents, we assigned the one that occupied rank size 4 to the X chromosome pair. In males, the sex bivalent invariably showed only one distal chiasma (Figure 1).

Statistical tests: The data were not normally distributed (Shapiro-Wilks *W*-test, $0 < P < 0.043$) and the samples were heteroscedastic (Levene test of homogeneity of variances, $P = 0.006$) and unbalanced (from 1 to 92 cells per individual and from 2 to 29 individuals per locality). For these reasons, nonparametric Mann-Whitney *U*-tests were used to compare mean values of chiasma scores between samples according to sex and race. In addition, the distribution of chiasmata along chromosomal arms, divided into 10 segments, was compared between samples using chi-square tests. All tests were performed with Statistica 4.3 (StatSoft, Tulsa, OK). Corrections for multiple tests were made using the sequential Bonferroni tests (Dunn-Sidak method, see Sokal and Rohlf 1995, p. 241). The probability values provided in Tables 2 and 5 are those corrected according to this procedure.

RESULTS

Chiasma number: No differences in chiasma counts were present between localities within each race (Mann-Whitney *U*-test, Rb males, $P = 0.186$; standard males, $P = 0.248$; Rb females, $P = 0.315$; all standard females were from Kairouan). Thus, data were pooled between localities within races in subsequent analyses. In addition, as chiasma number (CN) is known to decrease with age, particularly in female mice (Polani and Jagiello 1976; Speed 1977) correlations between age and CN were calculated. Results indicated that, although a similar trend was observed, the effect was not significant either in males (Pearson: $r = -0.012$, $P = 0.852$ in Rb, range 72–698 days and $r = -0.082$, $P = 0.338$ in standard mice, range 78–594 days) or in females (Pearson: $r = -0.257$, $P = 0.066$ in Rb, range 55–608 days and $r = -0.011$, $P = 0.940$ in standard mice, range 41–599 days).

Chiasma scores were compared between sexes within each race. Mean cell CN was significantly higher in females than in males in both races (Table 2). As only one chiasma was present on the XY bivalent in males, but likely more than one on the X chromosome pair in females due to its large size (Mather 1938), the contribution of the sex chromosomes to this difference between sexes was estimated. The autosomal CNs of standard females using the X-removal method differed from that including the X bivalent ($P = 0.012$). Likewise, the CN values with and without the X bivalent were significantly different in Rb females ($P = 0.005$). The within-race comparisons excluding the sex chromosomes are presented in Table 2. When the sex chromosome contribution was removed from the data, the mean number of chiasmata between male and female standard mice was no longer significantly different, but remained so between male and female Rb mice. In all subsequent analyses, autosomal CN values are provided excluding the presumptive sex bivalent.

As shown in Table 2, standard mice presented significantly more chiasmata per bivalent arm than did Rb mice, regardless of sex and with or without the sex bivalent. The number of chiasmata per cell ranged from 21 to 29 in standard females, from 19 to 31 in standard males, from 19 to 26 in Rb females, and from 19 to 24 in Rb males. The CN value of an average autosomal arm bivalent was 1.28 (±0.09) and 1.21 (±0.08) in standard females and males, respectively, and 1.17 (±0.08) and 1.07 (±0.01) in Rb females and males.

Localization of chiasmata: The mean distribution of chiasmata per cell along autosomal arms is presented per sex and race in Figure 2, in which single and multiple chiasmata are differentiated. The observed CN per distance class is recorded in Tables 3 and 4. Results clearly showed an overall nonrandom distribution that was similar between sexes and races. On average, although chiasmata were present along the whole chromosomal arm,

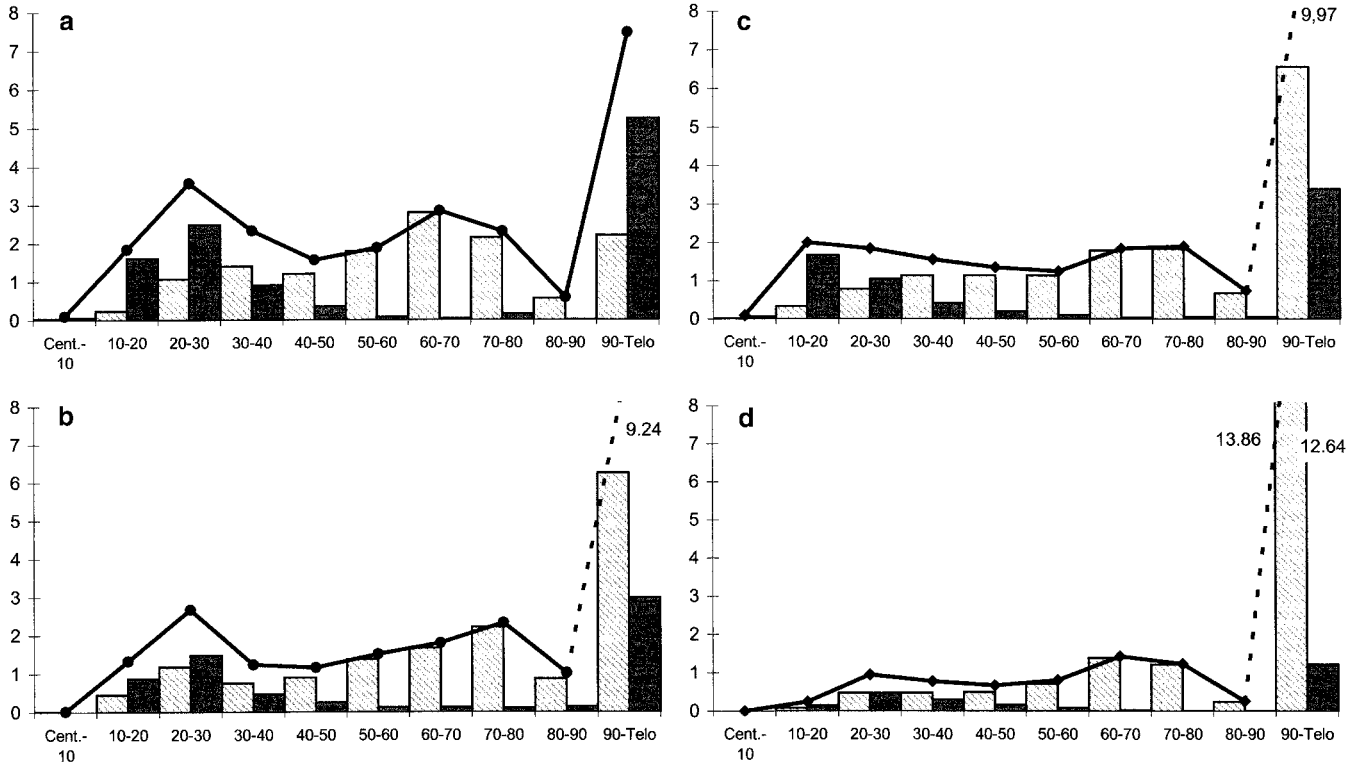

FIGURE 2.—Mean chiasma distribution per cell along autosomal arms in females (a) $2N = 40$ and (b) $2N = 22$ and males (c) $2N = 40$ and (d) $2N = 22$. Single chiasmata are represented by hatched bars, multiple ones by solid bars, and their sum by a solid line.

a preferentially terminal location was observed where 52% of all chiasmata occurred. In nonterminal regions, the distribution was roughly bimodal with a low number of chiasmata in an interstitial position and an even lower one in the centromeric and subterminal segments. Generally, when only one chiasma occurred per bivalent arm, its location was preferentially on the distal half of the arm (84%) or more specifically on the terminal region (54%). Almost all multiple chiasmata were double ones, a maximum of three being observed in both standard and Rb individuals in only seven bivalents. In bichiasmate arms, one chiasma was generally located proximally and the second distally, most frequently (96%) in a terminal position.

If the general pattern of distribution was similar between sexes and races, differences in the mean number of chiasmata per class and type were apparent between groups. Males and females significantly differed in the distribution of single, multiple, and total chiasmata (chi-square tests, all $P < 0.001$). In each race, females showed a significantly higher number of nonterminal chiasmata compared to males due to an increase in the number of both multiple and single chiasmata in this class (Mann-Whitney *U*-tests, all $P < 0.001$; see also Figure 2, a *vs.* b and c *vs.* d). As observed between sexes, differences between races within each sex were highly significant, whether the overall distribution (chi-square tests, $P < 0.001$ for all but one, for which $P = 0.016$) or the terminal and nonterminal classes ($P < 0.001$ in all Mann-Whitney *U*-tests) were considered (Figure 2, a *vs.* c and b *vs.* d). The general pattern observed was a decrease in nonterminal chiasmata, particularly in the proximal region, and an increase in the mean number of terminal chiasmata in Rb mice compared to standard mice. As a minimum of one chiasma per arm is required for proper chromosomal segregation, the change in position of single chiasmata in Rb mice can correspond only to a shift from a nonterminal to a terminal location. Similarly, the decrease in multiple chiasmata in Rb individuals occurred with a preferential loss of the proximal chiasmata within proximal-distal pairs resulting in the retention of a single chiasma in a terminal position. In conclusion, the reduction in CN in Rb mice was related to a decrease in multiple chiasmata (−2.44 and −2.13 per cell in males and females, respectively) and a shift of single chiasmata from a nonterminal to a terminal position (−3.76 and −1.84, respectively); both of these modifications led to an increase in the frequency of terminal chiasmata (+6.07 and +4.03, respectively).

The 19 and X bivalents: Similar comparisons were performed for the shortest autosome, ranked 19, the only one not involved in an Rb fusion. In only seven cases were multiple chiasmata scored for this chromosome, none of which were present in Rb males (Tables 3 and 4). Thus, the CNs were similar in all samples and did not significantly differ from one per bivalent (Mann-

TABLE 3

Distribution of the number (and percentage) of chiasmata per chromosomal segment in female mice

	Standard (21 individuals/51 cells)						Rb (26 individuals/55 cells)					
	Single			Multiple			Single			Multiple		
	Autosomes	Chrom. 19	Chrom. X	Autosomes	Chrom. 19	Chrom. X	Autosomes	Chrom. 19	Chrom. X	Autosomes	Chrom. 19	Chrom. X
Cent.–10	2 (0.3)	0 (0)	0 (0)	3 (0.5)	0 (0)	1 (1.7)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
10–20	12 (1.7)	1 (2)	0 (0)	82 (14.6)	0 (0)	12 (20)	25 (2.9)	0 (0)	3 (7.7)	48 (13.3)	0 (0)	7 (21.9)
20–30	55 (8)	1 (2)	4 (19)	127 (22.7)	2 (50)	13 (21.7)	65 (7.5)	2 (3.8)	8 (20.5)	82 (22.7)	3 (50)	3 (9.4)
30–40	72 (10.4)	3 (6.1)	3 (14.3)	47 (8.4)	0 (0)	3 (5)	42 (4.9)	3 (5.8)	6 (15.4)	26 (7.2)	0 (0)	3 (9.4)
40–50	62 (9)	3 (6.1)	3 (14.3)	18 (3.2)	0 (0)	1 (1.7)	50 (5.8)	3 (5.8)	3 (7.7)	14 (3.9)	0 (0)	1 (3.1)
50–60	92 (13.3)	6 (12.2)	4 (19)	4 (0.7)	0 (0)	0 (0)	76 (8.8)	8 (15.4)	6 (15.4)	7 (1.9)	0 (0)	2 (6.3)
60–70	143 (20.7)	15 (30.6)	1 (4.8)	2 (0.4)	0 (0)	1 (1.7)	92 (10.6)	6 (11.5)	3 (7.7)	7 (1.9)	0 (0)	0 (0)
70–80	110 (15.9)	9 (18.4)	6 (28.6)	8 (1.4)	0 (0)	2 (3.3)	122 (14.1)	9 (17.3)	5 (12.8)	6 (1.7)	0 (0)	0 (0)
80–90	29 (4.2)	1 (2)	0 (0)	1 (0.2)	0 (0)	0 (0)	48 (5.5)	1 (1.9)	1 (2.6)	8 (2.2)	0 (0)	2 (6.3)
90–Telo	113 (16.4)	10 (20.4)	0 (0)	268 (47.9)	2 (50)	27 (45)	344 (39.8)	20 (38.5)	4 (10.3)	164 (45.3)	3 (50)	14 (43.8)
Total	690	49	21	560	4	60	865	52	39	362	6	32

The number of single and multiple chiasmata is provided for all autosomes, for bivalent 19 alone, and for the X bivalent.

Whitney *U*-tests, $0.45 < P < 0.91$). However, the distribution of chiasmata along the chromosome was different in males and females within races (chi-square test, Rb, $P = 0.013$; standard, $P = 0.002$), but not between races within sexes (chi-square test, males, $P = 0.056$; females, $P = 0.127$). Comparisons involving the X bivalent in females showed a significant difference in mean cell CN between races (standard, 1.57 ± 0.42; Rb mice, 1.25 ± 0.34; Mann-Whitney *U*-test, $P = 0.013$), but not in the overall localization of these chiasmata (chi-square tests, $P = 0.880$, 0.446, and 0.685 for single, multiple, and total chiasmata, respectively).

Interference: In bichiasmate arms, the mean distance between two chiasmata, *i.e.*, chiasma interference, ranged from 67.2% ± 12.7 to 75.6% ± 11.7 of the length of the arm (Table 5). A very significant difference was observed between races, Rb mice revealing a smaller average interference distance than that of standard mice in both sexes (see tests in Table 5). Differences between sexes within races were also significant, males showing a higher average interference distance in the standard race, whereas females did in the case of Rb mice.

In Rb mice, the existence of a chiasma interference acting across centromeres (Colombo and Jones 1997; Broman and Weber 2000) and resulting in a negative correlation between the distance to the centromere of the most proximal chiasmata on each arm of a Rb fusion was investigated. No significant correlation was observed in our data for any of the five distance classes tested, that is, from 40 to 80% of the arm length between chiasmata (Pearson: $-0.73 < r < 0.19$, all $P > 0.05$). These results indicated that the centromere probably acted as a barrier to chiasma interference in these Rb fusions, as previously observed by Maudlin and Evans (1980), suggesting the independence of chiasma formation between Rb arms.

The mean centromere-to-chiasma distance was significantly higher in Rb mice than in standard ones in the case of single chiasmata. The same trend was observed for the mean distance between the centromere and the most proximal component of multiple chiasmata, but only comparisons among males were significant (Table 5).

DISCUSSION

Chiasma distribution patterns: This study represents the most extensive report on chiasma distribution patterns in wild male and female house mice. Our results on CN for all-acrocentric individuals from two localities in Tunisia are in agreement with previous data for standard laboratory mice from various strains, which reported a mean number of 20.9–23.9 chiasmata per spermatocyte and 23.8–28.9 per oocyte (Polani 1972; Speed 1977; Jagiello and Fang 1987; Lawrie *et al.* 1995). However, a major finding of this study is the existence of a significant decrease in CN in male and

TABLE 4

Distribution of the number (and percentage) of chiasmata per chromosomal segment in male mice

	Standard (10 individuals/195 cells)				Rb (10 individuals/229 cells)			
	Single		Multiple		Single		Multiple	
	Autosomes	Chrom. 19	Autosomes	Chrom. 19	Autosomes	Chrom. 19	Autosomes	Chrom. 19
Cent.–10	4 (0.1)	1 (0.5)	14 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
10–20	65 (2.2)	1 (0.5)	325 (23.5)	2 (50)	20 (0.5)	0 (0)	36 (6.4)	0 (0)
20–30	154 (5.1)	8 (4.1)	205 (14.8)	0 (0)	110 (2.7)	3 (1.3)	109 (19.3)	0 (0)
30–40	223 (7.4)	15 (7.8)	81 (5.9)	0 (0)	110 (2.7)	13 (5.7)	69 (12.2)	0 (0)
40–50	224 (7.4)	14 (7.3)	39 (2.8)	0 (0)	115 (2.8)	11 (4.8)	38 (6.7)	0 (0)
50–60	222 (7.4)	9 (4.7)	22 (1.6)	0 (0)	164 (4)	12 (5.2)	21 (3.7)	0 (0)
60–70	350 (11.6)	20 (10.4)	9 (0.7)	0 (0)	320 (7.9)	16 (7)	8 (1.4)	0 (0)
70–80	358 (11.9)	5 (2.6)	12 (0.9)	0 (0)	279 (6.9)	10 (4.4)	3 (0.5)	0 (0)
80–90	133 (4.4)	2 (1)	12 (0.9)	0 (0)	57 (1.4)	0 (0)	3 (0.5)	0 (0)
90–Telo	1281 (42.5)	118 (61.1)	663 (48)	2 (50)	2894 (71.1)	164 (71.6)	279 (49.3)	0 (0)
Total	3014	193	1382	4	4069	229	566	0

The number of single and multiple chiasmata is provided for all autosomes and for bivalent 19 alone.

female Rb mice compared to all-acrocentric individuals, similar to that observed in two recent analyses restricted to males (Bidau *et al.* 2001; Castiglia and Capanna 2002).

The analysis of chiasma patterns indicates that nonterminal, particularly proximal, chiasmata are less frequent and distal ones are more numerous in Rb than in standard mice. These changes are related to a significant reduction in the number of double chiasmata, in which the proximal component is most frequently lost, whereas the distal one is maintained, contributing to the considerable increase in single terminal chiasmata observed in Rb mice (Tables 3 and 4; Figure 2). Similarly, results show that in multiple chiasmata, the mean distance to the centromere of the proximal component is longer in Rb mice (30.3%) compared to standard individuals (24%; $P < 0.001$, see Table 5). These combined results suggest that the chiasma-suppressing effect related to the centromere, *i.e.*, centromere interference (Beadle 1932; Choo 1998), may be higher in Rb than in $2N = 40$ individuals. The existence of a higher centromere interference is expected to affect single chiasmata in a manner similar to that observed for multiple ones. As predicted, Rb mice show significantly less nonterminal and more terminal single chiasmata than do standard individuals, suggesting that a shift from the former to the latter position has occurred. This reduction in nonterminal single chiasmata is also accompanied by a significant increase in their mean centromere-to-chiasma distance (72.1 and 85.2, respectively; $P < 0.001$, see Table 5). In addition, a derived effect of this centromere interference may be the significant decrease in chiasma interference, observed in Rb mice (from 74.9 to 68.2; $P < 0.001$, see Table 5).

These results indicate that formation of a centric fusion in the house mouse involves a more terminal redistribution of chiasmata, reducing the probability of formation of multiple chiasmata due to the combination of chiasma and centromere interference. The latter would be expected to be more pronounced in the proximal regions and decrease progressively toward the distal ends. Such a pattern is compatible with the observed increase in the distance to the centromere of chiasmata. As mice from these chromosomal races are similar genetically, but highly differentiated by the presence of Rb fusions (Saïd and Britton-Davidian 1991), the decrease observed may be related to the difference in chromosomal structure. Support for the relation between chromosomal structure and chiasma distribution is suggested by chromosome 19, which is the only autosome not involved in an Rb fusion and for which no modification in CN was observed between races.

Meiotic constraints: Previous studies have provided estimates of recombination rates in laboratory and wild mice carrying Rb fusions. However, few of these have analyzed homozygous Rb individuals, the main focus having been the evaluation of genic recombination in chromosomally heterozygous individuals (Polani 1972; Cattanach 1978; Maudlin and Evans 1980; Davisson and Akeson 1993; Bidau *et al.* 2001; Castiglia and Capanna 2002). These studies show that Rb heterozygotes generally exhibit crossover suppression in the proximal regions of the meiotic trivalents. This effect was ascribed to mechanical incompatibilities between the acrocentric and metacentric homologs, leading to a pairing delay of the synaptonemal complex and a lower probability of crossover formation in the pericentromeric regions (Davisson and Akeson 1993). In this case, as such structural incompatibilities are unlikely, a relationship between chromosomal structure and chiasma distribution must involve other meiotic constraints.

In house mice, the formation of a centric fusion re-

TABLE 5

Mean values (and standard error) for different interference distances in mice

Interference distance	Mean distance values						*Z*values of Mann-Whitney test					
	Standard			Rb			Between races			Between sexes		
	Females	Males	All 40	Females	Males	All 22	F40 *vs.* F22	M40 *vs.* M22	All 40 *vs.* all 22	F40 *vs.* M40	F22 *vs.* M22	
Between chiasmata	73.4 (±10.2)	75.6 (±11.7)	74.9 (±11.3)	69.9 (±12.9)	67.2 (±12.7)	68.2 (±12.8)	−2.74*	−10.70***	−10.59***	−4.54***	−2.43*	
Centromere to single chiasmata	62.5 (±23.4)	74.2 (±26.5)	72.1 (±26.4)	73.4 (±26.7)	87.8 (±21.4)	85.2 (±23.1)	−8.80***	−24.03***	−21.12***	−11.30***	−17.56***	
Centromere to multiple proximal	25.2 (±9.4)	23.5 (±11.0)	24.0 (±10.6)	27.3 (±11.5)	32.3 (±12.3)	30.3 (±12.2)	−1.47 ns	−11.45***	−10.48***	−4.07***	−4.86***	

Chiasma interference refers to the distance between two chiasmata on a chromosomal arm. Centromere interference refers to the distance between the centromere and a chiasma. Multiple proximal corresponds to the most proximal component of multiple chiasmata. Interference distances are expressed in percentage of chromosomal arm length in standard females and males (F40 and M40, respectively), in Rb females and males (F22 and M22), and in all standard and Rb individuals (All 40 and All 22). *Z*values of Mann-Whitney *U*-tests are indicated with corrected levels of significance: *$P < 0.05$; ***$P < 0.001$; ns, $P > 0.05$.

sults in the loss of a small amount of centromeric material, corresponding to the telomeres of both acrocentrics and to a variable amount of minor satellite DNA sequences, leaving the major satellite of both acrocentrics intact (Garagna *et al.* 1995). While heterochromatin is known to suppress recombination of chromosomal segments in its vicinity (John and King 1985), centric heterochromatin has also been shown to buffer the chiasma-suppressing effect of the centromere on adjacent euchromatin. Thus, removal of centric heterochromatin may lead to a decrease in proximal chiasmata (Yamamoto and Miklos 1978). An alternative mechanical constraint may be related to the fact that transition from an acrocentric to a metacentric structure is known to modify the spatial arrangement of chromosomes during the prophase of meiosis, in which chromosomes attach to the inner nuclear membrane by their telomeric regions and cluster in a restricted area of the nuclear surface (John 1990). In the case of Rb fusions, the centromere will no longer show a close spatial association with the nuclear membrane (Capanna and Redi 1994; Schertan *et al.* 1996). If this change in configuration modifies the chiasma maturation process (Roeder 1990; Maguire 1995; Zickler and Kleckner 1999), a redistribution of tension along the chromosomal arms may occur (Zickler and Kleckner 1998), favoring distal chiasma formation over proximal ones. Similarly, the transition from two acrocentrics, each with its own kinetochore, to a biarmed chromosome with only one kinetochore may result in a reduction in microtubule-capturing efficiency and tension maintenance per chromosomal length. If such a feature interacts with proximal chiasmata to increase aneuploidy rates due to chromosomal entanglement (Lamb *et al.* 1997), to tension imbalance during segregation (Sybenga and Rickards 1987; Nicklas 1997), or to premature loss of sister chromatin cohesion (Hawley *et al.* 1993; Moens and Spyropoulos 1995; Koehler *et al.* 1996), distal chiasmata may be selected for. The existence of a relation between chromosome structure and chiasma distribution suggests that this may be a general characteristic common to metacentric chromosomes. Although few comparative studies exist, a similar trend in which metacentric chromosomes show less overall and/or proximal chiasmata than do acrocentric ones has been observed in other species such as humans (Laurie and Hultén 1985), *Drosophila melanogaster* (Nachman and Churchill 1996; True *et al.* 1996), grasshoppers (Colombo 1993), plants (Parker 1987), and experimental yeast constructs (Kaback *et al.* 1992).

Sex differences and genic effects on recombination: Differences in chiasma counts and location between sexes have previously been observed in various laboratory strains of the house mouse (Polani 1972; Polani and Jagiello 1976; Jagiello and Fang 1987; Gorlov *et al.* 1994; Lawrie *et al.* 1995), although the magnitude of the intersex differences in CN depended on the strain studied (Speed 1977). Our results, which provide the

first data for wild mice, largely confirm this trend in both races studied: females show more chiasmata, located less terminally than in males, and thus agree with a higher recombination rate in the former than in the latter (Polani 1972; Speed 1977; Jagiello and Fang 1987). The reasons for these sex differences are under debate and two main theories have been proposed, one related to selection for reduced recombination in the heterogametic sex bivalents with a pleiotropic effect on autosomes and the other to sex-specific costs and benefits (see Burt *et al.* 1991; Korol *et al.* 1994).

That recombination in both sexes may be subjected to selective pressures of various origins is suggested by the chiasma patterns in Rb and standard individuals. In the latter, the difference in CN between the XX and XY bivalents largely contributes to the sex differences, whereas these involve both autosomes and sex bivalents in the Rb race. Previous studies have reported the absence of a significant difference in CN between the autosomes of male and female standard mice, although the CN tended to be larger in females than in males (Gorlov *et al.* 1994; Lawrie *et al.* 1995). However, in all strains examined, the location of chiasmata was always found to differ significantly between sexes, whether data were recorded on autosomal or whole cell bivalents (Polani 1972; Speed 1977; Jagiello and Fang 1987; Gorlov *et al.* 1994; Lawrie *et al.* 1995). Gorlov *et al.* (1994) demonstrated that this difference in chiasma distribution was sufficient to cause a difference in recombination rates between sexes, even in the absence of a sex difference in CN. In this study, the differences in CN observed between sex bivalents within and between races require confirmation by unambiguous identification of the X chromosome using specific probes (Hultén *et al.* 1995) and/or genic-based recombination estimates (Soriano *et al.* 1987).

The existence of selective constraints on recombination patterns between sexes suggests an alternative nonstructural hypothesis consisting in the independence between the occurrence of Rb fusions and chiasma patterns. In this case, reduced recombination rates would have been selected for in mice that carried Rb fusions. Due to disjunctional constraints, this can occur only through a decrease in the number of multiple chiasmata and/or a shift of chiasmata from a nonterminal position to a more terminal one, which decreases the fraction of genes exchanged (Korol *et al.* 1994). Such features would be compatible with the patterns observed in the Rb mice, as all bivalents including the sex ones would be expected to be affected in both sexes. However, if this were the case, there would be no reason to expect a decrease in the number of chiasmata specifically located in the centromeric region.

Thus, our data are more compatible with an increase in centromere interference in metacentric chromosomes *vs.* acrocentric ones leading to an overall decrease in the number of chiasmata, although genic factors are most likely involved in patterning chiasmata between sexes. Further analyses in additional races carrying less Rb fusions are required to confirm the absence of an interchromosomal effect on non-Rb chromosomes, particularly since chromosome 19, the only acrocentric autosome tested, may be too small to allow for a significant difference in the number of chiasmata to be observed (Mather 1938; Kaback 1996). However, a case in point is the study of Bidau *et al.* (2001) who show a similar alteration of chiasma distribution in wild males from Scotland homozygous for one to four Rb fusions. These modifications are restricted to the Rb fusions, chiasma patterns remaining unchanged in the acrocentric complement of the Rb mice.

Evolutionary implications: Whatever the mechanism involved in reducing CN, the change in chromosomal structure in Rb mice is associated with a generalized decrease in recombination. This is achieved through the combination of three factors: (i) the reduction in diploid number, which decreases interchromosomal recombination; (ii) the lower CN, which decreases intrachromosomal recombination; and (iii) the higher number of terminal chiasmata, which leads to an exchange of shorter DNA fragments, reducing the efficiency of recombination. Such modifications in recombination rate are expected to have an important effect on genic variability. This can be approximated by estimating the differential production of potential gametic combinations between races. The reduction in diploid number alone results in a $2^9 = 512$ times higher loss of gametic combinations in Rb mice ($2^{11} = 2048$ different combinations) compared to standard ones ($2^{20} = 1{,}048{,}576$). When the difference in the number of chiasmata is included by considering that each chiasma creates two independent chromosomal fragments, the difference between races increases to $2^{11.37} = 2647$ (Rb mice, 32.70; standard mice, 44.07), both sexes combined. In addition, if all terminal chiasmata are excluded due to a presumed limited effect on the efficiency of recombination, the mean number of 7.87 recombined arms is obtained for Rb mice and 13.72 for standard mice, which decreases the number of potential gametic combinations to $2^{14.85} = 29{,}532$ times less in the former than in the latter.

Are these differences in recombination rates adaptive and have they resulted in modifications of genic diversity patterns? Various theoretical models have investigated the conditions under which different levels of recombination will be selected for (Feldman *et al.* 1980; Sharp and Hayman 1988; Zhivotovsky *et al.* 1994; Otto and Michalakis 1998; Lenormand and Otto 2000 and references therein), as well as the relationship between genic variability and recombination rates (Charlesworth 1996; Nordborg *et al.* 1996; Nachman 2001 and references therein). From these studies, several somewhat simplistic predictions can be made, suggesting that a decrease in recombination rate will be

favored the less heterogeneous the environmental selection under specific epistatic values, the shorter the generation time, or the less intense the sib competition estimated by litter size. Similarly, a positive correlation between genic diversity of neutral or near neutral markers would be expected. Several biological parameters are available for the chromosomal races in Tunisia, allowing us to discuss their relevance to adaptive changes in recombination patterns. Although populations of both races occupy commensal habitats, Rb populations in Tunisia are exclusively restricted to the medina centers of cities, whereas standard mice are distributed at the periphery and in nonurban habitats (Saïd and Britton-Davidian 1991; Chatti *et al.* 1999). The localized distribution of the Rb populations suggested that they may have evolved specific adaptations to a high-density type of habitat, which were supported by changes in life history traits, Rb individuals showing significantly smaller litter sizes than those of standard mice in laboratory crosses (Saïd *et al.* 1993). In addition, estimates of allozymic diversity indicated a loss of variability in the Rb samples from Djemmal and Monastir compared to the standard ones (Saïd and Britton-Davidian 1991). However, recent studies have not confirmed the difference in litter size between races (K. Benzekri and N. Chatti, personal communication) and have further suggested that the reduction in diversity is most likely the result of a unique founder event following which further gene flow between races was impeded by the chromosomal barrier (Chatti *et al.* 1999). This is supported by an allozyme analysis of the two races in Kairouan, which, while showing the habitat segregation, had similar levels of heterozygosity (I. Ould Brahim, N. Chatti, J. Britton-Davidian and K. Said, unpublished observations). This is also the case for most European Rb races (including Scottish mice) in which allelic diversity and heterozygosity values match those in adjacent standard populations (Britton-Davidian *et al.* 1989). However, Riginos and Nachman (1999) have indicated a decrease in genetic variability of centromerically located microsatellite markers in several Rb populations, although a causal relationship to deterministic or stochastic forces could not be assessed. Thus, so far, convincing evidence is lacking for an environmental selective advantage of the decrease in recombination rates in Rb mice.

However, the present chiasma analysis predicts that fixation of Rb fusions in house mice should result in a rapid decrease in recombination rates. Previous studies have shown that this effect extends as well to chromosomally heterozygous individuals (Cattanach 1978; Davisson and Akeson 1993; Bidau *et al.* 2001), suggesting that the flow of genes from standard to Rb mice should be severely limited in proximal chromosomal regions (Castiglia and Capanna 2002). The role of chromosomal rearrangements in contributing a barrier to gene flow between populations and/or species through their effect on recombination patterns has been argued by a number of authors (Qumsiyeh 1994; Trickett and Butlin 1994; Britton-Davidian 2001) and recently highlighted in several studies (Noor *et al.* 2001; Rieseberg 2001). Further experimental and theoretical studies targeting genomic compartments according to their recombination rates and gene content (Eyre-Walker 1993; Nordborg *et al.* 1996; Zickler and Kleckner 1999; Nachman 2001; Petes 2001) are required to estimate the effect Rb fusions have on levels of genic diversity within races and on gene flow between them.

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